

herewith a PTO Form 1449 listing these documents and request that the Examiner return a signed copy of said PTO Form 1449 as evidence of her consideration.

In addition, Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the Sequence Listing. Support for all of the sequences listed in the Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the Sequence Listing, the corresponding computer-readable Sequence Listing, and the amendments submitted herewith.

Prior to the present invention, it was not known that HLA-G proteins could be expressed in other cells than cytotrophoblastic cells of the placenta (see pages 3-4 of the present application). Furthermore, as demonstrated in the attached publication of Real et al (*Journal of Reproductive Immunology*, 1999), although mRNA from the different HLA-G isoforms (-G1, -G2, -G4, -G3 and -G5) could be detected in tumor cell lines of different origin, no HLA-G protein expression could be detected in any of these tumor cell lines using 2 different monoclonal antibodies (87G and 01G anti-HLA-G1) (see, for example, page 268 under the heading "Results"). These analyses performed on a total of 26 tumor cell lines from different origin tested by immunohistochemistry and flow cytometry, revealed no HLA-G protein expression (see Table I of Real et al). The absence of HLA-G1 expression is consistent with other reports as described on page 271, end of the 1st paragraph, referencing a publication by Frumento et al. Therefore, Real et al concludes that given the low level of HLA-G mRNA that cannot be detected by mAb, compared to the high level of HLA-A, -B and -C expression (several magnitudes of difference) which are easily detected (see page 272 of Real et al).

The present invention is based on the discovery that the unconventional HLA class I antigen HLA-G, which has been shown to be essentially expressed by the cytotrophoblastic cells of the placenta and is considered to protect said trophoblast cells from the lytic activity of the NK cells from the decidual layer of the maternal endometrium, is expressed by some solid tumor cells (for example melanoma cells) as a biologically active protein that protects said tumor cells from NK-mediated cell lysis, resulting in escape of said tumor cells from elimination by the immune system. (See page 6, prenumbered line 4 through page 7, line 30; including the Table).

As shown in Figure 4, HLA-G expression profile differs depending on the tumor cells; noting that IGR melanoma cells express HLA-G2, -G3, and -G4 but not HLA-G1; M74 melanoma cells express HLA-G1 only; and M8 cells not expressing HLA-G. In addition, as shown in Figure 6 and 7, high level expression of these isoforms is correlated with the malignancy of the tumor and therefore is predictive for monitoring the evolution of the tumor; particularly noting the low level expression of HLA-G transcripts in healthy skin and healthy lymph node, high level expression in primary tumors and lymph node metastases, and decreased level of expression in regressing tumors. Likewise, Figure 8 demonstrates that no HLA-G protein expression in the same healthy skin sample was observed, whereas positive staining for HLA-G is expressed with monoclonal antibody W6/32 (anti- β M-associated HLA-class I α chain), 87G (anti-HLA-G) and 16G1 (anti-HLA-G soluble form).

As shown in Figures 5A through 5E, the expression of one of these HLA-G isoforms protects target cells from lysis by NK-cells resulting in escape of the tumor cells from elimination by the immune system.

Therefore, to solve the problem of monitoring and treating solid tumors, the Applicants have provided a method that establishes the HLA-G proteins expression profile of said tumors using antibodies specific for HLA-G membrane-bound and soluble isoforms, as claimed in Claims 2 and 3.

The rejection of the claims under 35 U.S.C. § 112, second paragraph is believed to be obviated by amendment.

The rejection of Claims 2 and 3 under 35 U.S.C. § 103(a) over Smith in view of Klein et al is respectfully traversed.

Smith discloses a method of distinguishing fetal cells from maternal cells by detecting HLA-G mRNA in said fetal cells using hybridization methods whereby the absence of hybridization indicates that the cells are maternal cells, whereas the presence of hybridization indicates that the cells are fetal cells. See, for example, column 8, lines 23-64.

Smith teaches that HLA-G is a fetus-specific histocompatibility gene whose mRNA is expressed only in the placenta and in few tissues in the fetus; it is not expressed in adult T cells and B cells lines while low levels of an alternatively spliced form of HLA-G have been found in adult lymphocytes. See column 1, lines 15-50 and column 3, lines 21-25. Therefore, Smith does not suggest the expression of an HLA-G protein in tumor cells, but rather Smith teaches that HLA-G expression is limited to few tissues in the fetus, to the placenta and to adult lymphocytes. Likewise, Smith also does not teach any function for the HLA-G protein since it uses only HLA-G mRNA as a fetal cell specific marker.

Klein et al discloses a method for the prognosis and the treatment of solid tumors, by measuring the level of expression of HLA class I antigens HLA-A, -B and -C using immunohistochemistry or by ELISA and anti- β 2M specific antibodies. See page 1239, 1st column. Klein et al also teach that HLA-A, -B and -C expression is required for the lysis of

malignant cells by the cytotoxic T cells and shows that a reduction in HLA class I expression is predictive of poor diagnosis and decreased survival and that an increase in HLA class I expression level in some patients may be of clinical value. See page 1242, column 2.

However, Klein et al do not suggest detecting different HLA class I antigen, e.g., HLA-G in solid tumors because Klein et al only disclose the detection of HLA-A, -B and -C antigens. Therefore, even in combination Smith and Klein et al provide no suggestion for establishing the expression of an HLA-G protein in tumor cells. In contrast, the teachings of these documents suggest that HLA-G expression is limited to the placenta. Applicants direct the Examiner's attention to MPEP § 2141.02: "PRIOR ART MUST BE CONSIDERED IN ITS ENTIRETY, INCLUDING DISCLOSURES THAT TEACH AWAY FROM THE CLAIMS." Nothing in these documents suggests any role of HLA-G in tumor immunity and as such a person of ordinary skill in the art would not be motivated from the teachings of Smith and Klein et al to establish the presence of an HLA-G protein in said tumor cells with a view to selecting treatment which is suited for the tumor or to monitor the evolution of a tumor. Therefore, the rejection under 35 U.S.C. § 103(a) is untenable and should be withdrawn.

Applicants submit that the present application is now in a condition for allowance.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Marked-Up Copy

Serial No: 09/622,583

Amendment Filed on:

OCTOBER 22, 2001

IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, one line before the title, please insert the following heading:

--TITLE OF THE INVENTION--

Between the title and line 4, please insert the following heading:

--BACKGROUND OF THE INVENTION--

FIELD OF THE INVENTION--

Between lines 8 and 9, please insert the following heading:

--DISCUSSION OF THE BACKGROUND--

Page 5, between lines 7 and 8, please insert the following heading:

--SUMMARY OF THE INVENTION--

Page 6, please replace the tables with the following new tables:

Primers	Nucleotide sequences	Hybridization temperatures	Isoforms amplified
G.257	5'-GGAAGAGGAGACACGGAACA (SEQ ID NO:1)	61	G1, G2, G3
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:2)		G4, G5, G6
G.526	5'-CCAATGTGGCTGAACAAAGG (SEQ ID NO:3)	61	G1, G4, G5

G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:4)		
G.-3-4	5'-ACCAGAGCGAGGCCAAGCAG (SEQ ID NO:5)	65	G3
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:6)		
G.-3	5'-ACCAGAGCGAGGCCAACCCC (SEQ ID NO:7)	65	G2, G6
G3.U	5'-GGCTGGTCTCTGCACAAAGAGA (SEQ ID NO:8)		
G.-3	5'-ACCAGAGCGAGGCCAACCCC (SEQ ID NO:9)	61	G6
G.i4b	5'-AAAGGAGGTGAAGGTGAGGG (SEQ ID NO:10)		
G.526	5'-CCAATGTGGCTGAACAAAGG (SEQ ID NO:11)	61	G5
G.i4b	5'-AAAGGAGGTGAAGGTGAGGG (SEQ ID NO:12)		

Probes	Nucleotide sequences	Hybridization temperatures (°C)	Isoforms
GR	5'-GGTCTGCAGGTTTATTCTGTC (SEQ ID NO:13)	60	HLA-G1, G2, G3, G4, G5, G6
G.647 F	5'-CCACCACCCTGTCTTTGACT (SEQ ID NO:14)	60	HLA-G1, G2, G5, G6
G.I4 F	GAGGCATCATGTCTGTTAGG (SEQ ID NO:15)	55	HLA-G5, G6
G.927 F	5'-ATCATGGGTATCGTTGCTGG (SEQ ID NO:16)	55	HLA-G1, G2, G3, G4, G5 and G6

Page 6, before prenumbered line 4, insert the following text:

--BRIEF DESCRIPTION OF THE DRAWINGS

-Figure 1 illustrates:

(A): the RT-PCR analysis of the HLA-G isoform mRNAs in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3' end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells and first trimester trophoblasts (TRO), and peripheral blood mononucleated cells (PBMC) were used, these cells being used as control cells for high transcription levels and basal transcription levels of HLA-G, respectively. IgR, M8, DRAN and M74 correspond to the amplification of the cDNA of melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe;

(B): this figure corresponds to the RT-PCR detection of alternative transcripts in melanoma cells. Primer 3 is specific for the HLA-G2 and soluble HLA-G2 (G6) isoforms which do not possess exon 3. Primer 3.4 makes it possible to distinguish the HLA-G3 mRNA transcripts. Primers G.526 and 14b amplify specifically the HLA-G5 transcript, which corresponds to the soluble form. The PCR products which were coamplified during the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe;

(C): this figure corresponds to the RT-PCR analysis of the HLA-G mRNA in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3'

end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells was used, these cells being used as control cells for high transcription levels. IGR, M8 and DRAN correspond to the amplification of the cDNA melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe.

- Figure 2 illustrates the RT-PCR analysis of the HLA-G isoform mRNAs in the biopsies of melanoma metastases (in vivo and ex vivo analysis of skin). The pan-HLA-G primers G.257 and 3G.U are used for the RT-PCR amplification of the HLA-G transcripts from skin metastases *ex vivo* (MEL) and from biopsies of healthy skin from the same patient (HS); JEG-3 cells and first trimester trophoblasts are used as controls (high level of HLA-G transcription). The HLA-G specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows.

- Figure 3 illustrates the detection of the HLA-G1 proteins in JEG-3 cells but not in IGR and M8 I melanoma cells, with the aid of the monoclonal antibody W6/32: the biotinylated surface proteins of melanoma and JEG-3 cells are immunoprecipitated using the monoclonal antibody W6/32; the immunoprecipitates are separated by SDS-PAGE at 12% and transferred onto cellulose membrane. The class I surface molecules are detected with streptavidin-conjugated peroxidase.

- Figure 4 illustrates the immunoprecipitation of the HLA-G isoforms of IGR melanoma cells with an antibody directed against the heavy chain of free HLA-G and with

the monoclonal antibodies 4H84 and HCA2. The cells are labelled for 30 min and immunoprecipitated with the specific antibodies, and the immunoprecipitates are analysed by SDS-PAGE at 10%. The antibody 4H84, which reacts with the HLA-G heavy chain (39-KDa band in JEG-3 cells), exhibits cross-reactions with the HLA-A, -B and/or -C heavy chains (45-KDa band in all the cells tested).

- Figure 5 illustrates:

(A): the effect of HLA-G expression in the IGR melanoma on sensitivity to lysis by the clone YT2C2-PR. K562 cells which are transfected either with the vector alone, or with the HLA-G1 vector containing the cDNA, or the HLA-G2 vector and the M8, M74, IGR and DRAN lines are used as target cells (T). The clone YT2C2-PR is used as an effector cell (E) in an effector cell/target cell (E/T) ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds 10% of the maximum release. This experiment is carried out at least 5 times and, each time, produces the same results;

(B): the inhibition of the lysis induced by the clone YT2C2-PR is due to an "off" signal which is transmitted by the IGR and DRAN cells. The M8 line is used as a target cell (T) and is chromium labelled. Clone YT2CT-PR is used as an effector cell (E) in an E/T ratio of 50:1. IGR and DRAM cells are added as inhibitor cells in an inhibitor cell/target cell ratio of 100, 50 and 25:1. 0 indicates that no IGR cell was added in the assay;

(C): the inhibition of the lysis induced by HLA-G-positive melanoma cells (target cells T). This figure illustrates more particularly the effect of HLA-G expression by IGR and DRAN melanoma cells on sensitivity to lysis by the clone YT2C2-PR. Several cell lines which are B-EBV, HLA-G negative [HOM (A3, B27, Cw1), BM (A29, B61 Cw2), SPO (A3, B7, Cw7), SWE (A2, B44, Cw5)] are lysed by the clone YT2C2-PR. This clone is used as an

effector cell (E) in an E/T ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds 10% of the maximum release;

(D) and (E): these figures show that the M8 HLA-G-negative tumour cells which are transfected with the cDNAs encoding the molecules G1, G2, G3 and G4 inhibit NK lysis (Figure 5E) and the cytotoxic T responses (Figure 5D). Figure 5D comprises, on the x-axis, the effector cells (E) (restricted HLA-A2 lines specific for an influenza peptide)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of specific lysis. The table below corresponds to the values obtained in this figure.

E/T ratio	M8-RSV	G1	G2	G3	G4	Genomic
15/1	55%	8%	39%	12%	17%	30%
7/1	52%	6%	42%	10%	14%	25%
3/1	29%	2%	30%	6%	12%	23%

- Figure 5E comprises, on the x-axis, the effector cells (E) (clone YT2C2-PR)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of-specific lysis.

- Figure 6 illustrates the detection of HLA-G transcripts in biopsies of human melanomas. The RT-PCR amplifications are carried out, using the abovementioned primers G.257 and G.3U, on biopsies of healthy skin (HS) and on healthy lymph nodes (HLN), on the one hand, and biopsies of lymph node metastases (LNM1 and LMN2). JEG-3 choriocarcinoma cells are used as control cells for high transcription levels. Specific HLA-G bands are revealed by hybridization with the GR-specific probe which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using the (3-actin primers are detected on the same membrane with the aid of β -actin probe.

- Figure 7 illustrates the RT-PCR analysis of the HLA-G transcripts in the biopsies of primary melanoma tumours and in the derived MPP5 primary cell cultures (*ex vivo* analysis). The abovementioned pan-HLA-G primers are used for the amplification from biopsies of healthy skin (HS1), from skin primary tumours (SPT1) and from tumours in regression (R1) which are obtained from the same patient, and from derived primary cells obtained from a skin tumour tissue (MPP5). The MPP5 cells and the SPT1 biopsy exhibit similar HLA-G transcription levels. JEG-3 cells are used as controls for high levels of HLA-G transcription. The HLA-G-specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using the β -actin primers are detected on the same membrane with the aid of a β -actin-specific-probe.

- Figure 8 illustrates:

(A) the specific detection of HLA-G5 transcripts by RT-PCR in biopsies of melanomas. The amplification of the HLA-G5 transcript from healthy lymph nodes (HLN), from a skin primary tumour (SPT1) and from two biopsies of lymph node metastases (LNM1 and LNM2) is carried out with the aid of the primers G.526 and G.i4b. The band corresponding to the HLA-G5 transcript is detected by hybridization with an I4F probe which is located in intron 4; JEG-3 cells are used as controls (high levels of HLA-G5 transcription). The band corresponding to the HLA-G5 transcript is indicated with arrows. The PCR products which were coamplified in the same reaction using the β -actin primers are- detected on the same membrane with a β -actin-specific probe;

(B) the immunohistochemical analysis of the soluble HLA-G expression in the LNM1 biopsy. Frozen and acetone-fixed sections of the LNM1 biopsy are positively stained with

the anti-melanoma antibody HMB45 (DAKO) and the anti-soluble HLA-G antibody 16G1, whereas the negative control gives no staining, using the Envision anti-mouse, peroxidase system (DAKO) and AEC as substrate.

DETAILED DESCRIPTION OF THE INVENTION--

Please delete, in its entirety, the text beginning at page 11, line 30, through page 16, line 28, indicated as follows:

[(A): the RT-PCR analysis of the HLA-G isoform mRNAs in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3' end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells and first trimester trophoblasts (TRO), and peripheral blood mononucleated cells (PBMC) were used, these cells being used as control cells for high transcription levels and basal transcription levels of HLA-G, respectively. IgR, M8, DRAN and M74 correspond to the amplification of the cDNA of melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe;

(B): this figure corresponds to the RT-PCR detection of alternative transcripts in melanoma cells. Primer 3 is specific for the HLA-G2 and soluble HLA-G2 (G6) isoforms which do not possess exon 3. Primer 3.4 makes it possible to distinguish the HLA-G3 mRNA transcripts. Primers G.526 and 14b amplify specifically the HLA-G5 transcript, which corresponds to the soluble form. The PCR products which were coamplified during

the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe;

(C): this figure corresponds to the RT-PCR analysis of the HLA-G mRNA in melanoma cells. pan-HLA-G primers [primer G.257 (exon 2) and 3G.U (untranslated 3' end)] are used for the PCR amplification of the HLA-G transcripts corresponding to the various known HLA-G isoforms. The cDNA from JEG-3 choriocarcinoma cells was used, these cells being used as control cells for high transcription levels. IGR, M8 and DRAN correspond to the amplification of the cDNA melanoma cell lines. The specific HLA-G bands are revealed by hybridization with the GR-specific probe, which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using β -actin primers are detected on the same membrane with the aid of a β -actin probe.

- Figure 2 illustrates the RT-PCR analysis of the HLA-G isoform mRNAs in the biopsies of melanoma metastases (in vivo and ex vivo analysis of skin). The pan-HLA-G primers G.257 and 3G.U are used for the RT-PCR amplification of the HLA-G transcripts from skin metastases *ex vivo* (MEL) and from biopsies of healthy skin from the same patient (HS); JEG-3 cells and first trimester trophoblasts are used as controls (high level of HLA-G transcription). The HLA-G specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows.

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and transferred onto cellulose membrane. The class I surface molecules are detected with streptavidin-conjugated peroxidase.

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- Figure 5 illustrates:

(A): the effect of HLA-G expression in the IGR melanoma on sensitivity to lysis by the clone YT2C2-PR. K562 cells which are transfected either with the vector alone, or with the HLA-G1 vector containing the cDNA, or the HLA-G2 vector and the M8, M74, IGR and DRAN lines are used as target cells (T). The clone YT2C2-PR is used as an effector cell (E) in an effector cell/target cell (E/T) ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds 10% of the maximum release. This experiment is carried out at least 5 times and, each time, produces the same results;

(B): the inhibition of the lysis induced by the clone YT2C2-PR is due to an "off" signal which is transmitted by the IGR and DRAN cells. The M8 line is used as a target cell (T) and is chromium labelled. Clone YT2CT-PR is used as an effector cell (E) in an E/T ratio of 50:1. IGR and DRAM cells are added as inhibitor cells in an inhibitor cell/target cell ratio of 100, 50 and 25:1. 0 indicates that no IGR cell was added in the assay;

(C): the inhibition of the lysis induced by HLA-G-positive melanoma cells (target cells T). This figure illustrates more particularly the effect of HLA-G expression by IGR and DRAN melanoma cells on sensitivity to lysis by the clone YT2C2-PR. Several cell lines which are B-EBV, HLA-G negative [HOM (A3, B27, Cw1), BM (A29, B61 Cw2), SPO (A3, B7, Cw7), SWE (A2, B44, Cw5)] are lysed by the clone YT2C2-PR. This clone is used as an effector cell (E) in an E/T ratio of 50/1. The results are expressed as the percentage of lysis recorded in 4 h in a chromium 51-release assay. Spontaneous release never exceeds 10% of the maximum release;

(D) and (E): these figures show that the M8 HLA-G-negative tumour cells which are transfected with the cDNAs encoding the molecules G1, G2, G3 and G4 inhibit NK lysis (Figure 5E) and the cytotoxic T responses (Figure 5D). Figure 5D comprises, on the x-axis, the effector cells (E) (restricted HLA-A2 lines specific for an influenza peptide)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of specific lysis. The table below corresponds to the values obtained in this figure.

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- Figure 5E comprises, on the x-axis, the effector cells (E) (clone YT2C2-PR)/target cells (T) (transfected M8 lines) ratios and, on the y-axis, the percentage of-specific lysis.

- Figure 6 illustrates the detection of HLA-G transcripts in biopsies of human melanomas. The RT-PCR amplifications are carried out, using the abovementioned primers G.257 and G.3U, on biopsies of healthy skin (HS) and on healthy lymph nodes (HLN), on the one hand, and biopsies of lymph node metastases (LNM1 and LMN2). JEG-3

choriocarcinoma cells are used as control cells for high transcription levels. Specific HLA-G bands are revealed by hybridization with the GR-specific probe which is located on exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using the (3-actin primers are detected on the same membrane with the aid of β -actin probe.

- Figure 7 illustrates the RT-PCR analysis of the HLA-G transcripts in the biopsies of primary melanoma tumours and in the derived MPP5 primary cell cultures (*ex vivo* analysis). The abovementioned pan-HLA-G primers are used for the amplification from biopsies of healthy skin (HS1), from skin primary tumours (SPT1) and from tumours in regression (R1) which are obtained from the same patient, and from derived primary cells obtained from a skin tumour tissue (MPP5). The MPP5 cells and the SPT1 biopsy exhibit similar HLA-G transcription levels. JEG-3 cells are used as controls for high levels of HLA-G transcription. The HLA-G-specific bands are revealed by hybridization with a GR-specific probe which is located in exon 2. The bands corresponding to the transcripts HLA-G1, G2, G3, G4 and G5 are indicated with arrows. The PCR products which were coamplified during the same reaction using the β -actin primers are detected on the same membrane with the aid of a β -actin-specific-probe.

- Figure 8 illustrates:

(A) the specific detection of HLA-G5 transcripts by RT-PCR in biopsies of melanomas. The amplification of the HLA-G5 transcript from healthy lymph nodes (HLN), from a skin primary tumour (SPT1) and from two biopsies of lymph node metastases (LNM1 and LNM2) is carried out with the aid of the primers G.526 and G.i4b. The band corresponding to the HLA-G5 transcript is detected by hybridization with an I4F probe which is located in intron 4; JEG-3 cells are used as controls (high levels of HLA-G5 transcription).

The band corresponding to the HLA-G5 transcript is indicated with arrows. The PCR products which were coamplified in the same reaction using the β -actin primers are- detected on the same membrane with a β -actin-specific probe;

(B) the immunohistochemical analysis of the soluble HLA-G expression in the LNM1 biopsy. Frozen and acetone-fixed sections of the LNM1 biopsy are positively stained with the anti-melanoma antibody HMB45 (DAKO) and the anti-soluble HLA-G antibody 16G1, whereas the negative control gives no staining, using the Envision anti-mouse, peroxidase system (DAKO) and AEC as substrate.]

Please replace the paragraph bridging pages 18 and 19 as follows:

--The specific HLA-G probes are as follows:

- GR, specific for exon 2,
- G.647 F (5'-CCACCACCCTGTCTTTGACT (SEQ ID NO:17): specific for exon 4),
- G.I4 F (GAGGCATCATGTCTGTTAGG (SEQ ID NO:18): specific for intron 4),

and

- G.927 F (5'-ATCATGGGTATCGTTGCTGG (SEQ ID NO:19): specific for exon

5).--

Page 19, line 5-12, please replace the paragraph with the following:

--The other probes are as follows:

- HLA-A-specific probe

(5'GGAGGACCAGACCCAGGACACG) (SEQ ID NO:20),

- HLA-B-specific probe

(5'AGCTCCGATGACCACAACCTGC) (SEQ ID NO:21)

- HLA-C-specific probe (5'TGTCCTAGCTGCCTAGGAG) (SEQ ID NO:22) and

- HLA-DRA-specific probe (TGTGATCATCCAGGCCGAG) (SEQ ID NO:23).--

Page 31 (Abstract), after the last line, beginning on a new page, please insert the attached Sequence Listing.

IN THE CLAIMS

--2. (Amended) A method [Method] for establishing the HLA-G expression profile of a solid tumour with a view to selecting a treatment which is suited to said tumour [and/or] or with a view to monitoring the evolution of said tumour, [characterized in that it comprises] comprising:

- (i) [the removal of] removing a tumour sample,
- (ii) [the preparation of] preparing a histological section from said sample,
- (iii) [the labelling of] labeling the cells of the sample obtained in (ii) with antibodies specific for HLA-G membrane-bound and soluble isoforms, and
- (iv) [the establishment of] establishing the HLA-G expression profile of said sample by detecting the labelled cells.

3. (Amended) A method [Method] for establishing the HLA-G expression profile of a solid tumour with a view to selecting a treatment which is suited to said tumour [and/or] or with a view to monitoring the evolution of said tumour, [characterized in that it comprises] comprising:

- (i) [the removal of] removing a tumour sample,
- (ii) optionally, [the labelling of] labeling the cells of said sample,
- (iii) [the lysis of] lysing the cells,
- (iv) [the bringing of] contacting the lysed cells [into contact] with various antibodies directed against the class I HLA antigens so as to possibly form HLA-G isoform/antibody complexes, and

(v) [the establishment of] establishing the HLA-G expression profile of said sample by detecting the complexes formed in step (iv).

Claims 14-23 (New).--